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MICROPHOTOMETRIC DNA ANALYSIS IN MODERATE DYSPLASIA OF THE UTERINE CERVIX

Correlation to the progression and regression of the lesion

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Abstract

Nuclear DNA content was determined by microphotometry in 27 cases of moderate dysplasia of the uterine cervix. All these cases were followed from one to ten years to study the biological behavior of moderate dysplasia in relation to cervical carcinogenesis. Ten of them showed progression to carcinoma in situ during a period of one to six years (median 34.8 months) and 17 cases showed regression to inflammation and normalcy during a period of one to ten years (median 44.2 months). DNA analysis was performed both on initial cervical smear and on representative smears collected during follow-up. Amongst ten cases which progressed to malignancy, nine had aneuploid DNA pattern and one had polyploid DNA pattern throughout the follow-up period. Amongst 17 cases which regressed to inflammation, 11 had euploid and six polyploid DNA pattern. The observation that moderate dysplasia with aneuploid DNA value often develops into carcinoma in situ in contrast to dysplasia with euploid or polyploid DNA value strengthens the conception that DNA aneuploidy may be considered as 'high risk indicator' for cervical precancerous lesions.

Key words: Uterine cervix, dysplasia, DNA ploidy.

Cancer of the uterine cervix is the most common malignant tumor among Indian women (1, 2). There is evidence that cancer of the uterine cervix does not arise from a normal epithelium but is preceded by a spectrum of abnormal epithelial changes most commonly termed 'cervical dysplasia' (3, 4). It has also been envisaged that some dysplastic lesions progress to malignancy or to higher grades of dysplasia while some persist and still others regress to normalcy (5-8). Microspectrophotometric studies of the DNA content in single cells of cervical smears are of high value in relating ploidy pattern to precancerous

and early cancerous lesions and their subsequent biological behavior (9-13). Some reports indicate that the degree of aneuploidy provides prognostic information useful for the management of the patient (14, 15). In the present study 120 cervical smears from 27 patients with moderate dysplasia were cytophotometrically examined and their DNA values were correlated with the biological behavior of the lesions.

Material and Methods

The material consisted of cervical smears from 27 women with the initial diagnosis of moderate dysplasia who were followed with repeated smear examination for periods ranging from 1 to 10 years (median = 42.1 months). Routine biopsy was not mandatory during the follow-up, but 50% of the dysplasia cases with long-term follow-up were subjected to biopsy on a random basis. Any women in the non-biopsy group showing higher grade of cytologic abnormality and/or clinical suggestion of a higher grade of lesion underwent biopsy. During follow-up 10 cases progressed to malignancy while 17 cases regressed to inflammation or normalcy.

Three to 7 representative cervical smears collected at initial examination and during subsequent follow-up visits were selected for DNA study. These smears were reviewed and dysplastic cells were marked by glass pencil. The coverslips were then removed and cells were destained,

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refixed in 10% neutral formalin, and processed for the Feulgen reaction (16). The DNA measurements were carried out using the Leitz' orthoplan with MPV compact standard outfit for absorption measurements with automatic data analysis system. In cases with cervical epithelial lesions 35 cells exhibiting morphologic signs of dysplasia or malignancy and 35 apparently normal intermediate cells were selected for measurement. In inflammatory smears benign epithelial cells (superficial and intermediate) were subjected to measurement. The DNA ploidy pattern described by Fu et al. (17) was adopted for this study.

Results

The DNA ploidy patterns during initial examination and the cytodiagnoses during subsequent follow-up visits are depicted in Table 1. Eleven of the 27 cases had euploid, 7 had polyploid, and 9 had aneuploid DNA value. All the 9 cases with aneuploid DNA pattern progressed to malignancy during follow-up periods ranging from 12 to 69 months (median 34.5 months) (Fig. 1). Of the 18 cases with euploid and polyploid pattern, only one case progressed to malignancy. The remaining 17 cases regressed to inflammation or normalcy during follow-up periods

Table 1
Cases of moderate dysplasia with their follow-up cytologic diagnosis and DNA ploidy pattern

Case No.	Age (years)	Initial cytologic diagnosis	Representative follow-up diagnosis (months)				DNA ploidy pattern
1.	27	Mod	Mod (15)	Mod (24)	Sev (27)	Mal (36)	Aneuploid
2.	39	Mod	Mod (24)	Mal (36)	—	—	Polyploid
3.	40	Mod	Mod (6)	Mal (18)	—	—	Aneuploid
4.	35	Mod	Sev (9)	Mal (21)	—	—	Aneuploid
5.	25	Mod	Mod (10)	Mal (36)	—	—	Aneuploid
6.	45	Mod	Mild (24)	Mild (36)	Mal (42)	—	Aneuploid
7.	40	Mod	Mod (12)	Sev (51)	Mal (69)	—	Aneuploid
8.	30	Mod	Mild (24)	Ml (51)	—	—	Aneuploid
9.	38	Mod	Sev (9)	Mal (12)	—	—	Aneuploid
10.	35	Mod	Mod (5)	Sev (7)	Mod (11)	Mal (26)	Aneuploid
11.	22	Mod	Mod (5)	Mod (12)	Inf (18–27) (2 s)	Inf (36)	Euploid
12.	30	Mod	Mild (2)	Inf (11)	Inf (15)	—	Euploid
13.	31	Mod	Mod (4–6)	Infl (8–24) (2 s)	Mod (38)	Inf (50–66) (2 s)	Polyploid
14.	40	Mod	Inf (4–16) (2 s)	Mod (23)	Inf (41–45)	Mod (51) Nor (72)	Polyploid
15.	24	Mod	Mod (2)	Inf (14–21) (2 s)	Inf (21–28)	—	Polyploid
16.	23	Mod	Mod (1)	Inf (9–12)	—	—	Polyploid
17.	35	Mod	Sev (6)	Inf (15–33) (2 s)	Mod (36)	Inf (42–9)	Polyploid
18.	28	Mod	Mod (3)	Inf (6–36) (2 s)	Dys (40)	Inf (65)	Euploid
19.	26	Mod	Mild (4)	Inf (12)	—	—	Euploid
20.	33	Mod	Mild (13–27) (2 s)	Inf (36–105) (3 s)	Nor (115)	—	Polyploid
21.	21	Mod	Inf (11)	Inf (28)	—	—	Euploid
22.	42	Mod	Inf (3–7)	Nor (17–28) (2 s)	—	—	Euploid
23.	26	Mod	Inf (2)	Inf (18)	Inf (18)	—	Euploid
24.	22	Mod	Inf (1)	Inf (6–54) (4 s)	—	—	Euploid
25.	32	Mod	Inf (20–48) (2 s)	—	—	—	Euploid
26.	25	Mod	Inf (6–40) (5 s)	—	—	—	Euploid
27.	35	Mod	Inf (18)	Nor (19–41) (2 s)	—	—	Euploid

Abbreviations: Nor = Normal; Inf = Inflammation; Mild = Mild dysplasia; Mod = Moderate dysplasia; Sev = Severe dysplasia; Mal = Malignant; (s) = Number of smears.

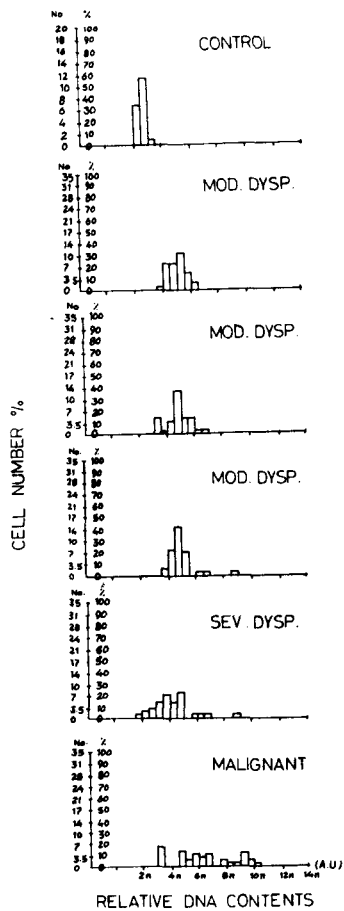


Fig. 1. DNA distribution pattern in control cells and cells cytologically diagnosed as moderate dysplasia, severe dysplasia and malignant (Case No. 1).

ranging from 12 to 115 months. These included 10 cases (Nos 11–20) which had either mild or moderate dysplastic changes at the first 2 or 3 follow-up visits and then regressed to inflammation (Fig. 2) and 7 cases (Nos 21–27) in which follow-up smears did not show any dysplastic changes after initial examination (Fig. 3). The difference in the rate of progression to malignancy between the aneuploid group and the polyploid/euploid group was highly significant ($p < 0.005$, Table 2).

Table 2

Correlation of DNA ploidy pattern with the biological behavior of moderate dysplasia

DNA ploidy pattern	Biological behavior		Total
	Progressed to malignancy	Regressed to inflammation/normalcy	
Aneuploid	9	0	9
Euploid/polyploid	1	17	18

$p < 0.005$ by Fisher's exact test.

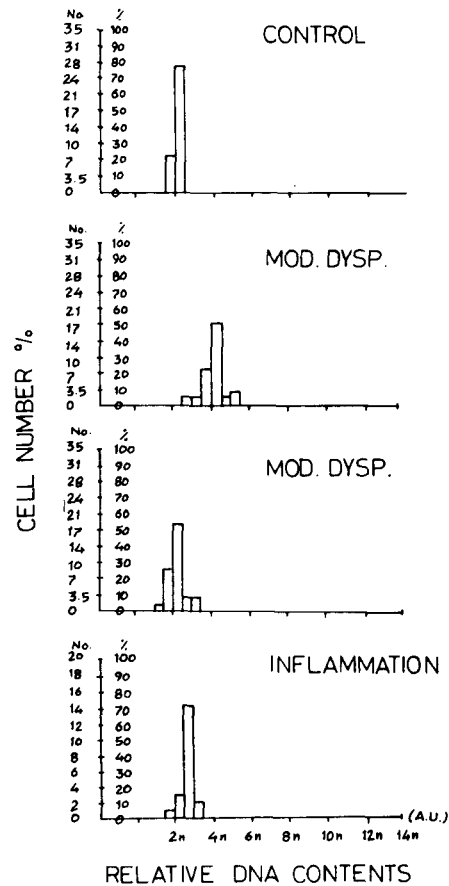


Fig. 2. DNA distribution pattern in control cells and in cells cytologically diagnosed as moderate dysplasia and inflammatory (Case No. 15).

Discussion

Although dysplasia of the uterine cervix has been considered a precursor of cervical cancer (4, 19), its biological nature is in many respects still unknown (20). Some dysplastic lesions progress to a higher grade of dysplasia or malignancy, some persist and still others regress to normalcy (5, 7, 8). An overall rate of progression from dysplasia to cancer of 4.4% to 65.5% has been reported, depending upon the severity of the lesions (5, 20). Epidemiological factors like young age at first sexual encounter and multiplicity of sexual partners have been found to be risk factors for progression of dysplasia to malignancy (21). DNA aneuploidy, Herpes simplex virus-2 infection and Human papilloma virus infection have also been reported as risk factors (18, 22, 23). In a number of studies it has been found that the DNA distribution in the tumor cells can be correlated to the prognosis (14, 15, 18, 24–26). Cervical epithelial lesions with aneuploid DNA pattern seem to have higher rates of persistence or recurrence than lesions with diploid and polyploid patterns (13, 18).

The present communication is based on an intermediate group of dysplasia, i.e. moderate dysplasia. This group was

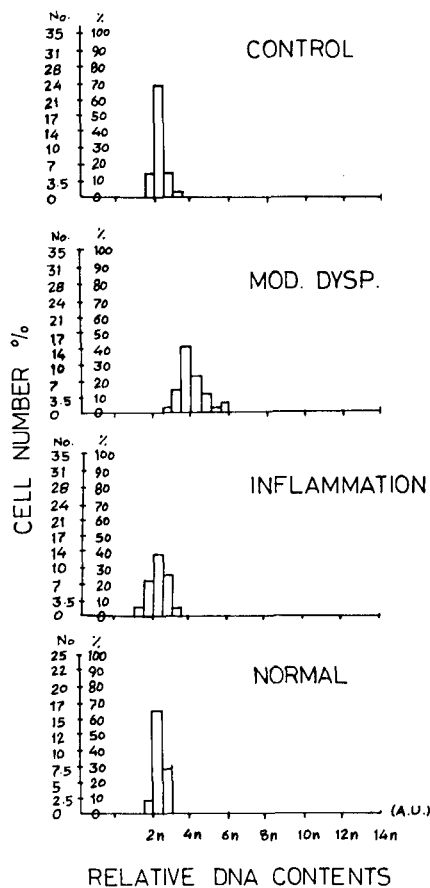


Fig. 3. DNA distribution pattern in control cells and cells cytologically diagnosed as moderate dysplasia, inflammatory and normal (Case No. 22).

selected for the study since it represents true dysplasia, i.e. neither a benign alteration resembling mild dysplasia nor carcinoma in situ (CIS), which at times is difficult to distinguish from severe dysplasia. An attempt has been made to correlate the biological behavior of the moderate dysplasia cases with their DNA ploidy pattern. When the cases of moderate dysplasia of the cervix were classified into two groups, viz those with aneuploid DNA pattern and those with polyploid or euploid DNA pattern, it was found that lesions with diploid or polyploid DNA value had a much better chance of regression than lesions with aneuploid pattern.

Our observation highlights the role of DNA ploidy pattern as an indicator for predicting the biological behavior of moderate dysplasia, our results being in contrast to those reported by Nasiell et al. (11), who did not find any remarkable relation between the DNA value of moderate dysplasia and progression or regression of the lesions. The result of the present study suggests that aneuploidy may be considered a 'high risk indicator' in cases with the initial cytologic diagnosis of moderate dysplasia.

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